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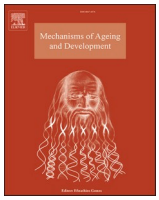

Genetic susceptibility to telomere shortening through the rs2293607 polymorphism is associated with a greater risk of alcohol use disorder

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ABSTRACT

Telomere shortening is usually considered a biomarker of ageing. Harmful alcohol use promotes accelerated biological ageing and alcohol use disorders (AUDs) are associated with short telomere length (TL). This study was conducted to examine the relationship of TL to AUD and determine whether single nucleotide polymorphisms (SNPs) in *TERC* and *TERT* modulate this association. For this purpose, we genotyped *TERC* SNPs rs2293607, rs12696304, and rs16847897 and *TERT* SNPs rs2735940, rs2736100, and rs2736098 in 308 male patients with AUD and 255 sex-matched healthy controls and measured TL in a subset of 99 patients and 99 controls paired by age and smoking status. Our results showed that the mean TL was shorter in patients with AUD than in controls. The area under the ROC curve was 0.70 ($P < 0.001$). The GG genotype of *TERC* rs2293607 was more common among patients with AUD than among controls (9.8% vs. 5.1%; $P = 0.038$). No difference was found for the other SNPs. Carriers of the GG genotype of rs2293607 had shorter telomeres than did allele A carriers. In conclusion, patients with AUD had shorter telomeres. Genetic susceptibility to telomere shortening through the rs2293607 SNP is associated with a greater risk of AUD.

1. Introduction

Telomeres are repetitive nucleotide sequences that protect the ends of chromosomes from deterioration (Wong and Collins, 2003). Telomere shortening occurs gradually with each round of cell division and consequently with increasing age; for this reason, the telomere length (TL) has been suggested as a marker of biological aging. In addition, telomere shortening may reflect cumulative damage (Blackburn et al., 2015), and is potentially associated with the etiology (Calado and Young, 2009) and prognosis (Perez-Rivera et al., 2014) of a great number of acute and chronic diseases.

Harmful alcohol use is associated with accelerated biological aging through several mechanisms like mitochondrial dysfunction (Leon et al., 2021) or monocyte methylation (Liang et al., 2022), which may partially explain the increased prevalence of ageing-related diseases in alcoholic patients (Safiri et al., 2022). Assessing the effect of alcohol on biological age is thus essential for understanding alcohol-related comorbidities. In this sense, TL studies focusing on alcohol consumption and alcohol use disorders (AUDs) have revealed no clear relationship between the TL and the alcohol intake quantity (Dixit et al., 2019), but growing

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evidence suggests an association between the presence of AUDs and the shortening of telomeres (Martins de Carvalho et al., 2019; Maugeri et al., 2021; Navarro-Mateu et al., 2021; Pavanello et al., 2011; Yamaki et al., 2019). Data on this topic are still scarce and controlling for all confounding factors is difficult, but certain genetic variants have been described as factors potentially able to modulate the association of the TL with AUDs (Do et al., 2015).

Thus, in this study we analyzed the relationship of the TL to AUDs. We also examined the potential modulating effects of several single nucleotide polymorphisms (SNPs) of the two subunits of the telomerase complex (*TERC* and *TERT*), which have been associated with human diseases and may modulate the TL: rs2293607, rs12696304, and rs16847897 of *TERC* and rs2735940, rs2736100, and rs2736098 of *TERT* (Jones et al., 2012; Matsubara et al., 2006; Zhang et al., 2012).

2. Material and methods

2.1. Study population

We collected peripheral blood samples from 308 male patients with AUDs from the Alcoholism Unit of the Internal Medicine Department of the University Hospital of Salamanca (Spain). All patients reported the daily consumption of more than 100 g ethanol (10 standard alcoholic drinks per day) for at least 10 years. Data on the patients' patterns of alcohol intake and the presence of liver cirrhosis were collected. According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria (APA, 2000), which were valid during the study recruitment period and applied in semi-structured interviews conducted by trained staff members, we classified the patients' AUDs as alcohol abuse or alcohol dependence. The Systematic Interview of Alcohol Consumption was used to quantify alcohol consumption (Gual et al., 2001). All subjects also underwent comprehensive psychiatric examination, and patients with addictions to other drugs (apart from nicotine) or major Axis I disorders (i.e., schizophrenia, mood disorders, or major anxiety disorders) were not included in the study. As detailed previously for this cohort (Marcos et al., 2008; Novo-Veleiro et al., 2018), the diagnosis of alcoholic cirrhosis was established by liver biopsy or by the presence of clinical, analytical, endoscopic, and/or radiological criteria in patients for whom biopsy was contraindicated. The absence of alcoholic liver disease (ALD) was confirmed using clinical, analytical, and radiological criteria. All patients were negative for hepatitis C and hepatitis B, and other causes of liver disease were ruled out.

A control group of 255 healthy male volunteers paired by age and smoking status was also enrolled. All of these individuals consumed less than 10 g ethanol per day, and neither they nor their first and second-degree relatives had a history of alcohol abuse or alcohol dependence.

All patients and control subjects were interviewed to ascertain that they were at least third-generation inhabitants of Castilla-León (northwestern Spain), and they provided informed consent to study participation. The study was carried out with the approval of the Ethics Committee of the University Hospital of Salamanca, and conformed to the tenets of the Declaration of Helsinki.

2.2. DNA extraction

Genomic DNA was extracted from nucleated peripheral blood cells using standard proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. The samples were stored at − 20ºC until use.

2.3. Telomere length analysis

For TL analysis, we selected from the cohort a sample of 198 middle-aged subjects (99 patients with AUD and 99 controls), paired according to age and smoking status. We included only subjects aged 40–75 years to minimize the influence of age on the TL (Blackburn, 2001). The patients in this sample had no active cancer. Smoking was defined as current or recent (in the last year) habitual tobacco consumption. Data on the presence of hypertension and diabetes were collected from medical reports. Cardiovascular disease was defined as a medical history of myocardial infarction or stroke.

After DNA extraction, the TL was measured by relative and comparative quantitative polymerase chain reaction (qPCR) that compares mean telomere repeat sequence copy number to a reference single copy gene. For each DNA sample, we performed six qPCR runs: three "T" runs (for the telomere repeat sequence copy number) and three "S" runs (for the single copy gene copy number). The reference single copy gene used in this study was 36b4. This method was initially described in 2004 (Gil and Coetzer, 2004) but employs the same primers previously developed in 2002 (Cawthon, 2002) and the 36b4 gene as an endogenous control.

The telomere-specific primers were: forward, 5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′ and reverse, 5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′. The 36b4-specific primers were: forward, 5′-CAGCAAGTGGGAAGGTGTAATCC-3′ and reverse, 5′-CCCATTCTATCATCAACGGGTACAA-3′. For each telomere amplification reaction, 10 ng DNA, 1 μl (5 μM) of the forward primer, and 2.33 μl (5 μM) of the reverse primer were used; for 36b4 gene amplification, 0.4 μl (5 μM) of each primer was used. For both reactions, 40 cycles were performed with an annealing temperature of 60 °C for 30 s.

Our PCR processes had an efficiency of 88.92% and determination coefficient of 0.976 for 36b4 gene amplification, and an efficiency of 76.67% and determination coefficient of 0.974 for telomere amplification. A standard curve derived from serially diluted reference DNA was generated for each T and S run. The average T/S ratio was calculated by dividing the average of the three T measurements by the average of the three S measurements.

2.4. Genotyping

The analysis of SNPs rs2293607, rs12696304, and rs16847897 of *TERC* and rs2735940, rs2736100 and rs2736098 of *TERT* was performed for the whole cohort and by allelic discrimination using real-time PCR (Jones et al., 2012; Matsubara et al., 2006). Samples from patients with and without ALD and controls were analyzed simultaneously on PCR plates to ensure that detection was blinded. The final volume of the real-time PCR reaction was 10 μl. The SNPs were genotyped using TaqMan MGB® assays for allelic discrimination with the StepOnePlus® System and TaqMan® Universal PCR Master Mix No AmpErase® UNG (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. The amplification program was performed with an annealing temperature of 60ºC for 1 min and 40 cycles.

2.5. Statistical analysis

Categorical variables were expressed as percentages of group members, and quantitative variables were expressed as means and standard deviations (SDs). Differences in categorical variables used to define baseline patient characteristics were studied using the χ^2 test. The Mann–Whitney *U* test was used to examine differences in quantitative variables. Results were deemed significant when the *P* value was ≤ 0.05. The statistical analyses were performed using IBM SPSS Statistics for Windows (version 25; IBM Corporation, Armonk, NY, USA).

2.5.1. TL analysis

A receiver operating characteristic curve was used to analyze differences in TL between patients with AUDs and healthy controls in order to quantify how accurately TL can discriminate between these two situations. Good correlation was defined as an area under the curve (AUC) ≥ 0.5. In addition, this analysis was used to choose the best cut-off point for patient classification according to the TL (the best cut-off has the

highest true positive rate together with the lowest false positive rate) since this is an objective and reproducible way to dichotomize a variable. We then performed a multivariate stepwise logistic regression analysis using this cut-off point to confirm the independent value of the TL in the presence of AUDs, including all potentially relevant confounding variables (hypertension, cardiovascular disease, smoking status, and cancer) in order to confirm the independent value of the TL in the presence of alcohol abuse or dependence. Alcoholic cirrhotic status was not included in the multivariable analysis due to the high risk of confounding and overfitting (this variable is directly related to alcohol consumption and was only recorded for cases).

In addition, we compared the TL by means of univariate analysis (Mann-Whitney U test) between patients with alcohol abuse and alcohol dependence due to the potential effect of alcohol pattern consumption in TL, and between cirrhotic and non-cirrhotic alcoholic patients. Finally, we sought to confirm the independence of the possible relationship between the TL and alcoholism by comparing the TLs of alcoholic cirrhotic patients and those without significant liver disease with those of paired healthy controls.

2.5.2. SNP analysis

Differences in allele and genotype frequencies between groups (alcoholic patients vs. controls and patients with ALD vs. those without ALD in order to analyze genetic susceptibility to AUDs and ALD, respectively) were compared using the χ^2 test or Fisher's exact test, as appropriate (expected frequency value < 5), and linear trend testing. The deviation of healthy controls' genotype frequencies from Hardy–Weinberg equilibrium was assessed using the χ^2 test.

Haplotype frequencies, haplotypic odds ratios (ORs), 95% confidence intervals (CIs), and pairwise linkage disequilibrium (LD) values were estimated. Haplotype frequencies were determined by means of the expectation-maximization algorithm (Tregouet et al., 2004), and LD values were calculated as Lewontin (D′) coefficients (Lewontin, 1988), with the THESIAS program (available at http://www.genecanvas.org). We also used this program to compare the distribution of the tested haplotype against a reference haplotype. To confirm the accuracy of haplotype calculations, we also performed haplotype analysis with Haploview 4.2 (available at http://www.broadinstitute.org/sections/science/programs/medical-and-population-genetics/haploview), (Barrett et al., 2005). Therefore, all haplotype analyses were conducted using both programs.

Finally, analysis of variance was used to assess the relationships among the genotypes of each SNP and TL. Tukey's honestly significant difference (HSD) test was performed to determine which genotypes were responsible for significant differences.

The statistical power of this study to detect an SNP conferring an OR of 2 for the presence of AUD was calculated using the Power and Sample Size Calculations software (version 3.0.43; http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize) (Dupont and Plummer, 1998). Assuming an α value of 0.05 and a prevalence of the least frequent allele among controls of 0.2, the statistical power for the entire study population was 95% (number of patients with alcohol use disorder, 308; number of healthy controls, 255).

3. Results

3.1. TLs of alcoholic patients and controls

The baseline characteristics of the cases and controls whose TLs were analyzed are shown in Table 1. We found no significant difference between patients with AUDs and healthy subjects in age or smoking status. According to the inclusion criteria, the control subjects had no significant pathology. According to the DSM-IV criteria, 60 (60.6%) patients had alcohol dependence and 39 (39.4%) patients had alcohol abuse. A total of 31 (31.3%) patients with alcohol abuse or dependence had alcoholic liver cirrhosis.

Table 1
Characteristics of cases and controls included in the telomere length analysis.

Variables	Cases (n = 99)	Controls (n = 99)	*P*
Age (years)	54 (11)	54 (12)	0.68
Smoking	87 (88.8%)	88 (88.9%)	0.98
Hypertension	17 (17.3%)	0	< 0.001
Cardiovascular disease	9 (9.2%)	0	0.002
Prior cancer	11 (11.2%)	0	0.001

Data are presented as mean (standard deviation) or absolute frequency (%).

The mean TLs (T/S ratios) were 4.38 (SD = 3.18) for patients with AUDs and 6.28 (1.94) for controls ($P < 0.001$). The AUC was 0.70 ($P < 0.001$; Fig. 1). The best cut-off point for the TL was 5.91 (70% sensitivity and 64% specificity); according to this threshold, the subjects were classified as having short and long telomeres. Among patients with AUDs, 69 (69.7%) individuals had short telomeres and 30 (30.3%) had long telomeres; 36 (36.4%) healthy controls had short telomeres and 63 (63.6%) had long telomeres ($P < 0.001$). Multivariable analysis using this cut-off point for the T/S ratio confirmed that only the presence of AUD was an independent risk factor for short telomeres (OR = 3.97; 95% CI, 2.19–7.18; $P < 0.001$) (Table S1).

In addition, no significant difference in TL was found between patients with alcoholic dependence and those with alcohol abuse, or between alcoholic patients with and without cirrhosis. The relationship between the TL and alcoholism was independent of the presence of cirrhosis: on average, telomeres were significantly shorter in alcoholic cirrhotic patients than in paired controls without AUD (5.68 [SD =3.98] vs. 7.21 [SD = 1.59]; $P = 0.03$) and in patients with AUD but no liver disease than in paired controls (4.22 [SD = 1.78] vs. 6.03 [SD = 1.47]; $P < 0.001$).

3.2. Associations of allelic variants in TERC and TERT with alcoholism and ALD

The distribution of the genotypes of SNPs rs2293607, rs12696304, and rs16847897 of *TERC* and SNPs rs2735940, rs2736100, and rs2736098 of TERT among control subjects were similar to those reported previously for Caucasians, and did not deviate significantly from Hardy–Weinberg equilibrium. The genotype distribution of the *TERC*

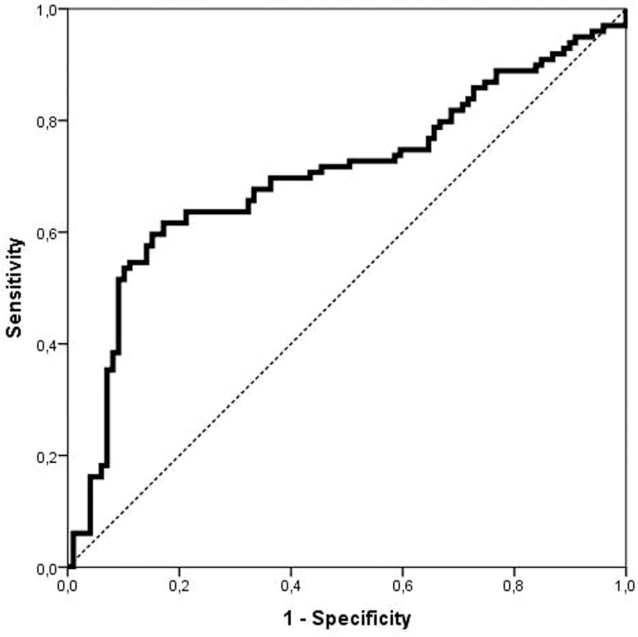

Fig. 1. Receiver operating characteristic curve for differences in telomere length between patients with alcohol use disorders and controls.

SNP rs2293607 differed significantly between patients with AUD and controls (Table 2). This difference was due to the presence of the GG genotype in a larger proportion of patients with alcohol abuse or dependence (9.8% vs. 5.1%; OR = 2.02; 95% CI, 1.03–3.95; $P = 0.038$). The genotype distribution of rs2293607 did not differ between alcoholic patients with and without liver disease. We found no significant difference in the genotype distributions of the other two SNPs of *TERC*.

We detected no significant difference in the genotype distribution of the *TERT* SNP rs2735940 between patients with AUDs and controls (Table 3). Nevertheless, the CC genotype was more prevalent among patients with AUDs and cirrhosis than among those without liver disease (25.7% vs. 14.7%; OR = 2.01; 95% CI, 1.11–3.64; $P < 0.020$). The genotype distributions of the other two SNPs of *TERT* did not differ significantly between patients and controls or between patients with and without liver disease.

Pairwise LD estimates obtained with Haploview showed a significant association among the three SNPs of *TERC* (Figure S1). These data did not differ from those obtained with THESIAS (rs2293607 - rs12696304: $D' = 0.83$, $P < 0.001$; rs2293607 - rs16847897: $D' = 0.67$, $P < 0.001$; rs12696304 - rs16847897: $D' = 0.70$, $P < 0.001$). Of the eight haplotypes of these SNPs identified with the THESIAS expectation-maximization algorithm, seven had frequencies > 1% in each group (Table S2). Relative to the reference haplotype (AGC), the haplotype GGC (corresponding to rs2293607, rs12696304, and rs16847897, respectively) was more common among patients with AUDs than among controls. In agreement with this result, Haploview analysis showed that the GGC haplotype was significantly more frequent among alcoholic patients ($\chi^2 = 24.24$, $P < 0.001$). The haplotype analysis revealed no significant difference between alcoholic patients with and without liver disease.

Table 2
Genotype and allele frequencies for *TERC* gene polymorphisms.

		Alcoholic patients vs. controls			ALC vs. AWLD		
		Alcoholics	Controls	*P*	ALC	AWLD	*P*
rs2293607	AA	163 (53.1)	156 (61.2)	0.048	52 (52.0)	105 (53.3)	0.490
	AG	114 (37.1)	86 (33.7)		35 (35.0)	75 (38.1)	
	GG	30 (9.8)	13 (5.1)		13 (13)	17 (8.6)	
	AA + AG	277 (90.2)	242 (94.9)	0.038[a]	87 (87.0)	180 (91.4)	0.238
	A allele	440 (71.7)	398 (78.0)	< 0.015	139 (69.5)	285 (72.3)	0.470
	G allele	174 (28.3)	112 (22.0)		61 (30.5)	109 (27.7)	
rs12696304	CC	19 (6.2)	20 (7.8)	0.661	6 (5.9)	13 (6.6)	0.243
	CG	120 (39.0)	93 (36.3)		46 (45.5)	70 (35.5)	
	GG	169 (54.9)	143 (55.9)		49 (48.5)	114 (57.9)	
	CC + CG	139 (45.2)	113 (44.1)	0.656	52 (51.4)	83 (42.1)	0.125
	C allele	158 (25.6)	133 (26.0)	0.900	58 (28.7)	96 (24.4)	0.251
	G allele	458 (74.4)	379 (74.0)		144 (71.3)	298 (75.6)	
rs16847897	CC	153 (49.7)	142 (55.7)	0.187	46 (45.5)	102 (55.8)	0.346
	CG	139 (45.5)	96 (37.6)		51 (50.5)	83 (42.1)	
	GG	16 (5.2)	17 (6.7)		4 (4)	12 (6.1)	
	CC+CG	292 (94.8)	238 (93.3)	0.459	97 (96)	185 (93.9)	0.440
	C allele	274 (44.5)	247 (48.4)	0.186	100 (49.5)	102 (50.5)	0.058
	G allele	342 (55.5)	263 (51.6)		163 (41.4)	231 (58.6)	

Data are presented as absolute frequency (%). Some subjects could not be genotyped for technical reasons. ALC, patients with alcoholic liver cirrhosis; AWLD, alcoholic patients without liver disease. aAA + AG vs. GG.

Table 3
Genotype and allele frequencies for *TERT* gene polymorphisms.

		Alcoholic patients vs. controls			ALC vs. AWLD		
		Alcoholics	Controls	*P*	ALC	AWLD	*P*
rs2735940	TT	92 (29.9)	67 (26.3)	0.387	27 (26.7)	63 (32.0)	0.066
	CT	158 (51.3)	129 (50.6)		48 (47.5)	105 (53.3)	
	CC	58 (18.8)	59 (23.1)		26 (25.7)	29 (14.7)	
	TT + CT	250 (81.2)	196 (76.9)	0.210	75 (74.3)	168 (85.3)	0.020[a]
	T allele	342 (55.5)	263 (51.6)	0.186	102 (50.5)	231 (58.6)	0.058
	C allele	274 (44.5)	247 (48.4)		100 (49.5)	163 (41.4)	
rs2736100	GG	78 (25.3)	60 (23.7)	0.568	22 (21.8)	53 (26.9)	0.529
	GT	158 (51.3)	124 (49)		53 (52.5)	102 (51.8)	
	TT	72 (23.4)	69 (27.3)		26 (25.7)	42 (21.3)	
	GG + GT	236 (76.6)	184 (72.7)	0.290	75 (74.3)	155 (78.7)	0.389
	G allele	314 (51.0)	244 (49.7)	0.359	97 (48.0)	208 (52.8)	0.269
	T allele	302 (49.0)	262 (50.3)		105 (52.0)	186 (47.2)	
rs2736098	AA	21 (6.8)	9 (3.6)	0.173	7 (6.9)	12 (6.1)	0.749
	AG	120 (39)	94 (37.2)		37 (36.6)	81 (41.1)	
	GG	167 (54.2)	150 (59.3)		57 (56.4)	104 (52.8)	
	AA+CG	141 (45.8)	103 (40.7)	0.228	44 (43.6)	93 (47.2)	0.550
	A allele	162 (26.3)	112 (22.1)	0.106	51 (25.2)	105 (26.6)	0.712
	G allele	454 (73.7)	394 (77.9)		151 (74.8)	289 (73.4)	

Data are presented as absolute frequency (%). Some subjects could not be genotyped for technical reasons. ALC, patients with alcoholic liver cirrhosis; AWLD, alcoholic patients without liver disease. aTT + CT vs. CC.

Pairwise LD estimates obtained with Haploview showed a significant association among the three SNPs of *TERT* (Figure S2). These data did not differ from those obtained with THESIAS (rs2735940 - rs2735940: $D' = -0.75$, $P < 0.001$; rs2735940 - rs2736098: $D' = -0.92$, $P < 0.001$; rs2736100 - rs2736098: $D' = 0.55$, $P < 0.001$). Of the eight haplotypes of these SNPs identified with the THESIAS expectation-maximization algorithm, six had frequencies > 1% in each group (Table S3). No significant difference in haplotypes was found between alcoholic patients and controls. Compared with the reference haplotype (CTG), the haplotype TGG (corresponding to rs2735940, rs2736100, and rs2736098, respectively) was more frequent among alcoholic patients without than among those with liver disease (Table S3). Nevertheless, Haploview analysis revealed no significant difference in this haplotype frequency between alcoholic patients with cirrhosis (0.218) and those without liver disease (0.282; $P = 0.093$).

3.3. Relationships of TERC and TERT SNPs to TL

Genotype variation in the *TERC* SNP rs2293607 was associated with differences in TL ($P = 0.012$; Table 4). Post-hoc comparison with Tukey's HSD test showed that carriers of the GG genotype of this SNP had shorter telomeres (4.41 [SD = 2.0]) than did AG carriers (5.87 [SD = 1.9]; $P = 0.014$) and AA carriers (5.82 [SD = 2.0]; $P = 0.012$); no difference was found between AG and AA allele carriers. Comparison of GG carriers with allele A carriers (AG and AA genotypes combined) also revealed a significant difference ($P = 0.003$). We found no significant relationship between the other two polymorphisms of *TERC* and the TL. The three polymorphisms of *TERT* were not related significantly to the TL, but carriers of the AA genotype of rs2736098 tended to have longer TLs (Table 5).

Table 4
Relationship between telomere length and *TERC* polymorphisms.

		N	Telomere length Mean (SD)	*P (ANOVA)*
rs2293607	AA	111	5.82 (2.0)	0.012
	AG	64	5.87 (1.9)	
	GG	19	4.41 (2.0)	
rs12696304	CC	15	4.95 (1.8)	0.279
	CG	72	5.63 (1.9)	
	GG	108	5.82 (2.1)	
rs16847897	CC	107	5.82 (2.0)	0.526
	CG	77	5.48 (2.0)	
	GG	77	5.75 (2.1)	

SD, standard deviation; ANOVA, analysis of variance.

Table 5
Relationship between telomere length and *TERT* polymorphisms.

		N	Telomere length Mean (SD)	*P (ANOVA)*
rs2735940	TT	59	5.60 (2.0)	0.830
	CT	88	5.63 (2.0)	
	CC	47	5.83 (2.2)	
rs2736100	GG	55	5.61 (2.0)	0.545
	GT	91	5.58 (2.0)	
	TT	49	5.96 (2.0)	
rs2736098	AA	13	6.03 (1.7)	0.647
	AG	74	5.53 (2.0)	
	GG	108	5.74 (2.1)	

SD, standard deviation; ANOVA, analysis of variance.

4. Discussion

Our detection of shortened telomeres in patients with AUDs relative to controls reinforces previous reports of the same finding (Martins de Carvalho et al., 2019; Pavanello et al., 2011; Yamaki et al., 2019). Although study design heterogeneity (including the use of different inclusion criteria, such as those based on the DSM-IV vs. DSM-5) are major problems, recent systematic reviews focusing on AUDs (Maugeri et al., 2021) and substance (mainly alcohol and cocaine) use disorders (Navarro-Mateu et al., 2021) have revealed that the condition of alcohol abuse, rather than the amount of alcohol consumed (Dixit et al., 2019; Latifovic et al., 2016), is associated with shorter TLs. At present, there is no clear explanation for these findings, but shorter TLs have also been associated with a wide range of psychiatric diagnoses, including depressive, psychotic, and anxiety disorders (Darrow et al., 2016).

In our cohort, carriers of the GG genotype of the *TERC* SNP rs2293607 had shorter telomeres on average than did allele A carriers for this genetic variation. The relationship of this allele to shorter telomeres in several populations has been reported (Do et al., 2015; Jones et al., 2012; Njajou et al., 2010), and functional in vitro data (Jones et al., 2012) showed that this SNP modulated expression levels of *TERC* mRNA with the A allele, producing longer telomeres. The likelihood that this SNP would influence TL in vivo is reinforced by the tagging of this allelic variant, located at 3q26.2, by the missense mutation rs10936599, which in turn has been associated with TL in a meta-analysis of genome-wide association studies (GWASs) (Codd et al., 2013).

Of note, we also found a greater prevalence of the GG genotype of rs2293607 of *TERC* among patients with AUDs than among controls. This finding was consistent with the greater frequency of a haplotype containing this G allele among patients with AUDs than among controls. No prior study of this SNP in subjects with AUDs or other neurological conditions has been reported, and no association with acute coronary syndrome was found in one of the few genetic association studies published (Perez-Rivera et al., 2015). Nonetheless, this variant and above-mentioned mutation rs10936599 have been associated clearly with susceptibility to several types of cancer in GWASs and genetic association studies (Chubb et al., 2013; Jones et al., 2012; Polat et al., 2019). Major depressive disorder (MDD) has also been associated with telomere shortening using a Mendelian randomization design (Michalek et al., 2017): T carriers for rs10936599 with MDD had shorter telomeres than did their CC-carrying counterparts and an increased risk of childhood-onset MDD compared with controls and adult-onset MDD.

We found a relationship between the CC genotype of the *TERT* SNP rs2735940 and cirrhosis, which could be in line with the described association of this polymorphism with other diseases, such as cancer (Liu et al., 2018). This association was not found in the haplotype analysis, and given the small sample used for this comparison, this result must be interpreted with caution.

Although our findings are limited by sample size, our results indicate that patients with AUD have shorter telomeres, consistent with previous findings, and that the rs2293607 variant likely modulates TL in vivo. The mechanisms through which the rs2293607 *TERC* variant and TL may impact the pathophysiology of AUDs, however, remain unclear. Genetic susceptibility to telomere attrition may favor low-grade inflammation and structural and functional brain changes that facilitate the development of AUDs, as other authors have suggested for mood disorders (Squassina et al., 2019). Conversely, external agents such as ethanol and psychosocial stress may interact to increase oxidative stress, promote inflammation, and induce hormonal, metabolic, and brain changes, which in turn may favor telomere shortening. For instance, childhood trauma was found to moderate the association between impulsive choice and TL among patients with alcohol dependence (Kang et al., 2017). The intriguing and complex association of telomere shortening with AUDs and other mental disorders has yet to be clarified. Although our results suggest a relationship between AUDs and biological ageing through genetic susceptibility to telomere shortening, this is only

a hypothesis and we need further studies to consider TL as a biomarker of ageing or a prognostic factor in alcoholic patients.

Several limitations should be considered in the interpretation of our results. First, telomere length in peripheral leukocytes could not reflect alterations in TL in other organs, although there are some studies that reveal a good correlation (Butler et al., 1998; Ma et al., 2016). Second, our results are only applicable to male patients since women were excluded in order to prevent gender from acting as a confounding factor due to both gender differences in ethanol effects and the ample evidence on the higher TL of females compared to males (Nawrot et al., 2004).

4.1. Conclusion

In summary, this study confirmed that patients with AUDs have shortened telomeres, which may be related to genetic predisposition through the rs2293607 SNP of *TERC*.

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Declaration of Competing Interest

No conflict declared.

Data availability

The authors are unable or have chosen not to specify which data has been used.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mad.2022.111693.

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